PB86-244308

Fluorescence Detection and Identification of Nitro Derivatives of Polynuclear Aromatic Hydrocarbons by On-Column Catalytic Reduction to Aromatic Amines

(U.S.) Environmental Sciences Research Lab.
Research Triangle Park, NC

1986

U.S. Department of Commerce
National Technical Information Service

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RE
EPA/600/J-86/120		PB86-244308

4. TITLE AND SUBTITLE
FLUORESCENCE DETECTION AND IDENTIFICATION OF NITRO DERIVATIVES OF POLYNUCLEAR AROMATIC HYDROCARBONS BY ON-COLUMN CATALYTIC REDUCTION TO AROMATIC AMINES

5. REPORT DATE
1986

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S) (Journal Version)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Sciences Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

10. PROGRAM ELEMENT NO.
C9YA1C-01 2077 (FY-84)

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Sciences Research Laboratory - RTP, NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/09

15. SUPPLEMENTARY NOTES
REF: Anal. Chem., 58(8):1827-1834, July 1986

16. ABSTRACT

Nitro-PAHs at trace level concentrations were reduced on-column to corresponding amino PAHs with a platinum/rhodium catalyst in a methanol-water reverse-phase HPLC system. The technique provides a convenient and sensitive method for detecting nitro-PAHs by fluorescence measurements of the corresponding amines. The technique has been incorporated in a simple automated system that eliminates sample cleanup in the determination of selected nitro-PAHs in complex matrices. Detection limit for 1-nitro pyrene is better than 10 pg on-column. Fluorescence spectra of 19 amino PAHs were measured. The catalyst does not change the chemical integrity of the PAHs but induces unknown chemical reactions on polar oxygenated species. The catalyst enhances the intensity of fluorescence emissions of most PAHs by removing quenching effects of dissolved oxygen. The pyrene emission signal is increased eightfold.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

Anal. Chem. 1986, 58, 1827–1834

EPA/600/J-86/120
JOURNAL ARTICLE

Fluorescence Detection and Identification of Nitro Derivatives of Polynuclear Aromatic Hydrocarbons by On-Column Catalytic Reduction to Aromatic Amines

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Nitro derivatives of polynuclear aromatic hydrocarbons (nitro-PAH) at trace-level concentrations were efficiently reduced to primary aromatic amines on a catalyst column packed with 5-μm alumina that was coated with platinum and rhodium (Pt/Rh). The reduction was accomplished in a reversed-phase HPLC system using methanol/water for the mobile phase. Reduction is classically catalytic; i.e., the Pt/Rh catalyst is not consumed. Fluorescence measurements permitted detection of some nitro-PAH as aromatic amines at low picogram levels. Excitation and emission wavelengths for fluorescence detection of 19 aromatic amines are given along with their fluorescence spectra. Some spectra exhibit unique features that are useful for confirming identification. An analytical system based on multidimensional HPLC principles was developed for routine analysis of specific nitro-PAH in complex organic matrices such as extracts of diesel exhaust particulates with minimal sample preparation.

Nitro derivatives of polynuclear aromatic hydrocarbons (nitro-PAH) have attracted considerable attention from a number of investigators during the last 7 years (*1–25*). These compounds have been shown to be direct-acting mutagens in the Ames *Salmonella* bioassay (*26*) and are easily formed by the reaction of PAH with oxides of nitrogen and nitric acid (*1, 11, 12*). The nitropyrenes in particular are the most mutagenic class of compounds reported in the literature (*4, 7*). Nitro-PAH have been observed in carbon black and toners (*3, 4*), in organic extracts of particles in ambient air (*2*), and in particulate exhaust emissions of diesel- and gasoline-powered engines (*5, 6, 14, 19, 20–24*). Results from some of these experiments suggest that nitro-PAH may account for a significant fraction of the direct-acting mutagenicities in the soluble organic fraction of these samples.

The presence of nitro-PAH in environmental samples is a legitimate cause for concern because some nitro-PAH or their amine derivatives are known carcinogens (*25, 27*). There is a need to identify and quantify these nitro-PAH to assess their potential health risk and their impact on the environment.

Several analytical techniques have been used to detect nitro-PAH. Among these are thin-layer chromatography with ultraviolet (UV) and fluorescence detection (*19, 20*) and high-performance liquid chromatography (HPLC) with detection by UV (*4*), fluorescence (*19, 22, 23, 28*), and mass spectrometry (*29*). HPLC with electrochemical detection has also been reported (*13, 28*). Capillary gas chromatography combined with mass spectrometry has been shown to be a very selective and sensitive analytical technique (*6, 30*). Unfortunately, the instrumentation is not readily available in most laboratories. Less expensive and simpler alternative chromatographic detection techniques are needed especially for routine monitoring applications. More recently, a number of selective GC detectors have been applied to nitro-PAH analysis (*31, 32*).

The sensitivity and selectivity of fluorescence measurements have been amply demonstrated in the analysis of certain classes of compounds such as the PAH (*33*). Although nitro-PAH generally do not fluoresce, most of them become highly fluorescent when reduced to aromatic amines. Hence the reduction of nitro-PAH to aromatic amines offers a convenient approach to the detection and identification of nitro-PAH by fluorescence measurements.

Noble-metal catalysts have been shown to reduce nitro-PAH to aromatic amines (*34*). Other catalyst systems combined with reducing agents have been described (*35*).

Previously, we developed an HPLC method for the determination of soluble sulfates in automotive exhaust emissions (*36*). In that method, an alcoholic solution of sulfates at sub and low microgram per milliliter levels is injected onto a column packed with crystalline barium chloranilate, and the released dye is measured by a UV monitor at 312 nm. We reasoned that a similar approach using a packed bed of noble-metal catalyst might reduce the nitro-PAH to aromatic amines and make in-line fluorescence detection possible. We reported the first successful application of this "on-column" reduction and fluorescence detection technique for nitro-PAH in 1981 (*22*).

This paper describes a fast and efficient catalytic method for reducing in-line, trace quantities of nitro-PAH to amino-PAH in a reversed-phase HPLC system using a methanol/water mobile phase. The reduction permits the detection and identification of some nitro-PAH by fluorescence measurements down to low picogram levels. A routine analytical system based on multidimensional HPLC principles (*37, 38*) has been developed to detect and/or quantitate specific nitro-PAH in complex organic matrices with very minimal sample preparation. The utility of the analytical system will be demonstrated in the determination of 1-nitropyrene in organic extracts of particles from exhaust emissions of gasoline- and diesel-powered vehicles.

MacCrehan and May (*28*) recently reported a similar approach for reducing nitro-PAH by using a column packed with a mixture of zinc powder and silica and a mobile phase consisting of acetonitrile and water buffered with ammonium acetate. However, they observed, that the "reducer" column has a finite active lifetime as the zinc is consumed in the reaction. In contrast, the catalyst described in this report has an indefinite lifetime and can function in the presence of binary solvents, water and methanol, without any solvent modifiers.

EXPERIMENTAL SECTION

Instrumentation. Two HPLC systems were used in this study. The first consisted of a Varian Model 8500 gradient liquid chromatograph, a Valco Instruments, Inc., standard HPLC six-port valve, a DuPont Zorbax ODS (4.5 mm i.d. × 250 mm) column that was protected by a guard column (2.1 mm i.d. × 50 mm) packed with Whatman pellicular ODS, a Perkin-Elmer Model MPF-44A fluorescence spectrophotometer equipped with a 20-μL flow cell, and an X–Y recorder. This system was used in the

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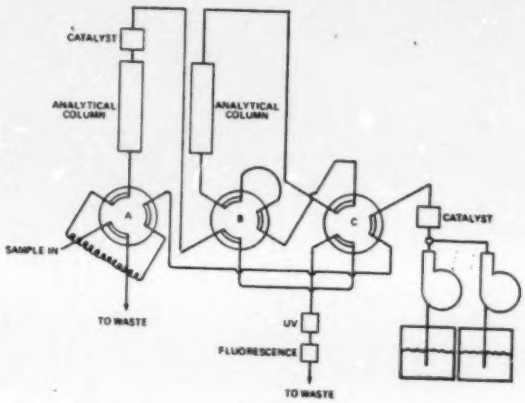

Figure 1. Schematic of the analytical system for routine analysis of specific nitro-PAH in complex organic matrix. See text for details.

evaluation of the catalyst materials for reducing nitro-PAH and in the development of a routine analytical method for specific nitro-PAH.

The second system is shown in Figure 1. The hardware consisted of a Varian Model 5000 liquid chromatograph, Valco Instruments, Inc., six-port HPLC valves, a Perkin-Elmer Model 650-S fluorescence spectrophotometer equipped with a 20-μL flow cell, a Waters Associate Model 440 absorbance monitor, a dual-channel analog recorder, and an electronic integrator. The analytical columns are identical DuPont Zorbax ODS (4.6 mm i.d. × 150 mm). Both catalyst columns (4.6 mm i.d. × 50 mm) are packed with 5-μm alumina that was coated with platinum and rhodium (Pt/Rh). The catalyst columns are wrapped with silicone-insulated heating tape and heated to about 80 °C. The switching valves, B and C, are pneumatically actuated via event timers available in the control module of the liquid chromatograph. These valves are used to direct the flow of the mobile phase for specified time intervals through one of the following column/detector configurations: (1) ODS–catalyst–detector as shown in Figure 1, (2) ODS–catalyst–ODS–detector, (3) ODS–detector, and (4) ODS–catalyst–detector. This particular configuration of the system components conveniently permits the selection of one or more eluant fractions from the catalyst column for further separation on the second analytical column.

Solvents and Chemicals. All organic solvents were "distilled-in-glass" UV grade (Burdick and Jackson Laboratories, Muskegon, MI) and were used as received. Water used in HPLC was charcoal-filtered deionized water.

1-Nitronaphthalene was obtained from Chem Service, West Chester, PA; 2-nitronaphthalene, 1,5-dinitronaphthalene, 6-nitrochrysene, and 6-nitrobenzo[*a*]pyrene from D. Schuetzle, Ford Motor Co., Dearborn, MI; 2-nitrofluorene, 2-aminoanthracene, and 1-nitropyrene from L. King, U.S. Environmental Protection Agency, Research Triangle Park, NC; 9-nitroanthracene, 2-nitroanthracene, 1-methyl-9-nitrophenanthrene, 3-nitrofluoranthene, 9-methyl-10-nitroanthracene, and 4-methyl-3-nitropyrene from Midwest Research Institute, Kansas City, MO; 1,3-, 1,6-, and 1,8-dinitropyrene, 1,3,6-trinitropyrene, and 1,3,6,8-tetranitropyrene from D. Sanders, Cabot Corp., Billerica, MA, and R. Mermelstein, Xerox Corp., Rochester, NY. 8-Nitrofluoranthene and nitrobenz[*a*]anthracene were synthesized by nitration of the parent PAH with concentrated nitric acid vapor, similar to the microsynthetic method described by Schuetzle (*6*).

Catalysts. The two catalyst materials that were found most effective in reducing nitro-PAH in methanol/water mobile phase contained Pt/Rh as active ingredients. The first of these was a prototype "three-way" catalyst (Englehard Industries, Edison, NJ) that was designed to reduce hydrocarbons in automotive exhaust emissions. It was ground, and particles between 20 and 100 μm were collected into four size ranges. Analytical catalyst columns (4.6 mm i.d. × 76 mm) were prepared by drypacking with 38–45-μm particles.

The second catalyst was Pt/Rh coated onto 5-μm Spherisorb 5AY alumina. Noble metals concentration was about 1%. This catalyst was prepared through the courtesy of I. Lichtenstein, Johnson Matthey, Inc., Malvern, PA. A column of this mate was prepared by drypacking and the bed compacted by pump 50% aqueous methanol at 10 mL/min. (**Caution:** Methanol m be flushed from the column with water every time an end fitti is removed. The catalyst was observed to flame spontaneou when wetted with methanol-rich aqueous solution and allow to dry in open air.)

Four other catalyst materials (zinc powder, 5% rhodium alumina, palladium on alumina, and rhenium metal powder) we obtained from commercial sources. Analytical columns (4.6 m i.d. × 76 mm) of these materials were prepared by drypackir

Reduction of Nitro-PAH. The reduction of nitro-PAH aromatic amines was accomplished by injecting a dilute soluti of the individual analytes onto an ODS analytical column co nected in series with a catalyst postcolumn. The elution w conducted isocratically with methanol/water mobile phas Nominal flow rate was 1 mL/min, and mobile phase compositi nominally ranged from 75 to 100% methanol. The effluent fro the catalyst column was monitored by a UV and a fluorescen detector in series. Sufficient material was initially injected give a readily measurable UV peak. The peak was later trapp in the flow cell of the fluorescence detector and both the excitati and emission spectra were obtained. A few of the catalyst effluer peaks were collected and submitted for analysis by solids prol mass spectrometry.

In the initial work, the "three-way" catalyst column was co nected after the ODS column and was immersed in a water bat Repetitive injections of 1-nitropyrene were made at vario catalyst temperatures (ambient to ~90 °C) while monitoring th aminopyrene response. The temperature above which no mo increase in aminopyrene response was observed was considere optimum temperature. The use of modifiers (HCl, NH_4OH NaOH) for the methanol/water system as well as acetonitrile water without modifiers was investigated under optimum tem perature conditions. The other catalyst materials were tested f their ability to reduce 1-nitropyrene using conditions found o timum for the "three-way" catalyst.

The efficiency of reduction of the nitro-PAH was estimate by using coupled-column configurations. Details are describe in the Results and Discussion Section.

Sample Collection and Preparation. Particles from th exhaust emissions of gasoline- and diesel-powered vehicles wer collected on Teflon-coated glass fiber filters using dilution tunn techniques and extracted with methylene chloride. Details ar described elsewhere (*40*). An aliquot of the extract was transferre to a tared vial, the solvent removed by blowing with a gentl stream of nitrogen and weighed. The solvent-free extract wa redissolved in a 50% solution of methanol in methylene chlorid to a nominal concentration of 2.5 mg/mL. This solution was use for routine determination of specific nitro-PAH (e.g., 1-nitro pyrene) without further treatment. All sample handling an analysis were performed under yellow light.

RESULTS AND DISCUSSION

Our first attempt to reduce nitro-PAH was done on a po stcolumn packed with ground "three-way" automotive catalys and with 1-nitropyrene as the test analyte. No measurabl fluorescence was observed in the absence of the catalyst, bu significant fluorescence was noted when the catalyst was i line. The elution volume of the fluorescent peak through th catalyst column was practically equal to the column voi volume. Other nitro-PAH were observed to behave similarl when monitored at the optimum excitation and emissio wavelengths of the corresponding amine derivative.

Stop-flow scanning techniques confirmed the reduction o the nitro-PAH. The fluorescence spectra of catalyst-reduce 2-nitroanthracene and 1-nitropyrene were identical to th fluorescence spectra of authentic 2-aminoanthracene an 1-aminopyrene, respectively. The spectra of catalyst-reduce 1- and 2-nitronaphthalene were similar to published spectra of 1- and 2-aminonaphthalene (*27*). Fluorescence spectra o 19 aromatic amines that were produced by in-line catalyti reduction were also obtained. Several compounds, such as 1-aminonaphthalene, 1,5-diaminonaphthalene, 3-amino-

Table I. Relative Retention Time (RRT) of Nitro-PAH on ODS-Catalyst Column, Wavelength Pairs, and On-Column Detection Limit for Fluorescence Detection of Corresponding Amino-PAH

compd	RRT[a]	wavelengths (excitation/emission), nm	detection limit,[b] pg
1,5-dinitronaphthalene	0.26	231/390	30
1-nitronaphthalene	0.33	243/429	4
2-nitrophthalene	0.36	234/403	14
1,3,6,8-tetranitropyrene	0.56	397/465	
1-nitrofluorene	0.56	285/370	14
9-nitroanthracene	0.58	263/505	34
2-nitroanthracene	0.70	260/495	16
1,3,6-trinitropyrene	0.77	396/460	
1,6-dinitropyrene	0.79	369/442	10
9-methyl-10-nitroanthracene	0.80	267/534	1000
1,8-dinitropyrene	0.89	395/454	50
1-methyl-9-nitrophenanthrene	0.91	254/440	50
8-nitrofluoranthene	0.99[c]	300/550	
1-nitropyrene	1.00[d]	360/430	10
3-nitrofluoranthene	1.05	300/530	80
1,3-dinitropyrene	1.08	395/445	
4-methyl-3-nitropyrene	1.19	355/420	12
6-nitrochrysene	1.53	273/437	47
x-nitrobenz[*a*]anthracene	1.50[e]	300/475	
6-nitrobenzo[*a*]pyrene	2.35	420/475	7

[a] Conditions: column, 15 cm × 4.6 mm i.d. Du Pont Zorbax ODS + 5 cm × 4.6 mm i.d. Pt/Rh column; mobile phase, 80% methanol in water; flow, 1 mL/min; detector, Perkin-Elmer Model 650-S fluorescence spectrophotometer. [b] Detection limit at S/N between 7 and 20. No values indicate that only qualitative standards were available. [c] Identification based on product abundance (ref 6) of nitration of parent PAH with vapors of concentrated HNO_3. [d] Retention time of 1-nitropyrene is 14.88 min. [e] Main nitration product using microsynthetic method (ref 6), nitro position undetermined.

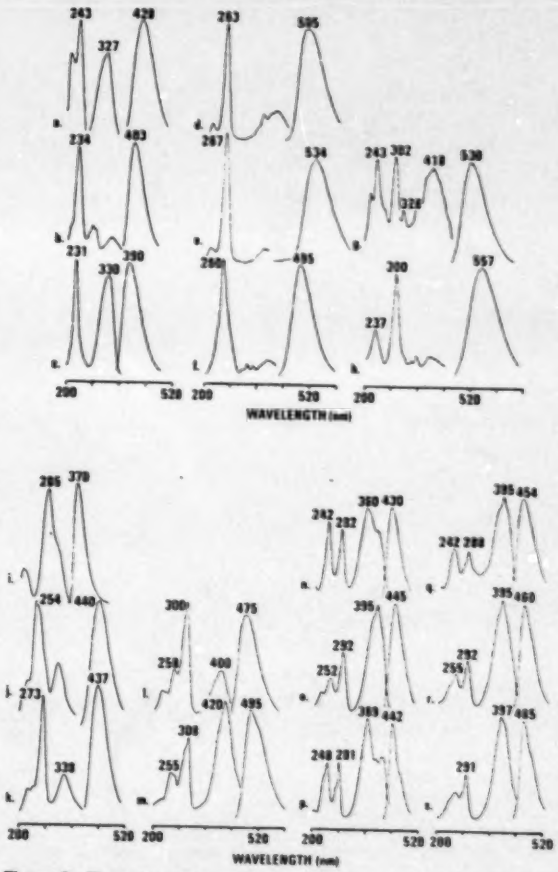

Figure 2. Fluorescence spectra of aromatic amines produced by in-line catalytic reduction of corresponding nitro-PAH. Compound identities are as follows: (a) 1-aminonaphthalene, (b) 2-aminonaphthalene, (c) 1,5-diaminonaphthalene, (d) 9-aminoanthracene, (e) 9-methyl-10-aminoanthracene, (f) 2-aminoanthracene, (g) 3-aminofluoranthene, (h) 8-aminofluoroanthene, (i) 2-aminofluorene, (j) 1-methyl-9-aminophenanthrene, (k) 6-aminochrysene, (l) *x*-aminobenz[*a*]anthracene (main nitration product, nitro position not known), (m) 6-aminobenzo[*a*]pyrene, (n) 1-aminopyrene, (o) 1,3-diaminopyrene, (p) 1,6-diaminopyrene, (q) 1,8-diaminopyrene, (r) 1,3,5-triaminopyrene, (s) 1,3,6,8-tetraaminopyrene. All spectra were measured in 80 or 90% aqueous methanol.

fluoranthene, 6-aminobenzo[*a*]pyrene, and the aminopyrenes, have several unique spectral features that are useful in confirming identification and might also prove analytically useful (Figure 2). The optimum wavelength pairs for detecting amino-PAH are summarized in Table I.

Additional confirmation of catalytic reduction was obtained by solids probe MS. The parent peaks of the following catalyst-reduced and unreduced species differed by 30 mass units: 1-nitropyrene, 2-nitroanthracene, 1-nitronaphthalene, 2-nitronaphthalene, 9-nitroanthracene, and 3-nitro-9-fluorenone. In the case of 2,7-dinitro-9-fluorenone, a difference of 60 mass units was observed between the parent peaks. These observed mass differences suggest that these nitro-PAH were converted to primary aromatic amines.

The "three-way" catalyst performed best when it was maintained at 60–80 °C. The addition of inorganic acid to the aqueous methanol mobile phase decreased the intensity of fluorescence emissions of the aromatic amines. In order to get reproducible fluorescence spectra of some amino-PAH, especially those containing two or more amino groups, it was found necessary to add ammonium hydroxide or NaOH to the water mobile phase to make it slightly basic (pH <8). However, the addition of ammonium hydroxide to the mobile phase resulted in gradual and irreversible loss of catalytic activity after about 2 months of daily use. Presumably this was due to a gradual depletion of the active catalyst materials via formation of soluble ammonia complexes. For this reason NaOH is preferred. The base is not really necessary for the reduction of nitro-PAH or for the fluorescence detection of the aromatic amines under flow conditions, but should be considered if spectral scanning of the aromatic amine peak is contemplated. The catalyst appeared to be active only in methanol/water mobile phase. No reduction was observed when the mobile phase was changed to acetonitrile/water.

The Pt/Rh on 5-μm spherical alumina was the best catalyst for reducing nitro-PAH. It contained more Pt/Rh per unit mass of substrate than the "three-way" catalyst and produced peaks with the least broadening largely due to the narrow size range of the substrate. A 5-cm column (4.6 mm i.d.) of the Pt/Rh catalyst was observed to be more reactive and had a higher reductive capacity than a 7.5-cm column (4.6 mm i.d.) of the "three-way" catalyst under the same elevated operating temperatures. This particular batch of the Pt/Rh catalyst was also observed to reduce nitro-PAH efficiently even at room temperature. Nonetheless, in practice, we used the optimum operating temperature (~80 °C) observed for the "three-way" catalyst. The catalyst is more likely to remain clean and reactive at elevated temperatures. Most of the results presented in this paper were obtained by using this catalyst.

The other catalyst materials were found inferior to the "three-way" catalyst. Acid-washed zinc powder reduced the nitronaphthalenes, 1-nitropyrene, and the nitro-9-fluorenones. Commercially available rhodium (5% on alumina) also reduced 1-nitropyrene. Neither palladium on alumina nor rhenium metal powder reduced 1-nitropyrene. It must be

emphasized that these materials were tested in methanol/water mobile phase only. No reagents were added to the mobile phase other than the sodium hydroxide used to increase the water pH between 7 and 8.

Both the Pt/Rh and the "three-way" catalysts have almost indefinite lifetime in methanol/water mobile phase. We have columns of both materials that are still active after almost daily use for 18 months. This strongly suggests that the reduction process is classically catalytic; i.e., the catalyst is not consumed. Pumping methylene chloride through the catalyst column for prolonged periods resulted in irreversible loss of catalytic activity. However, microliter amounts of methylene chloride, acetonitrile, dimethylsulfoxide, and hexane corresponding to normal sample injections during HPLC analysis did not have any deleterious effects on the performance of the catalyst.

The reduction of nitro-PAH through the ODS–catalyst columns is very reproducible. Six replicate manual injections of 500 pg of 1-nitropyrene gave a mean peak area of 154 388 μV-s and percent relative standard deviation of 0.9%.

We discovered during our first experimentations with the "three-way" catalyst the the catalyst was very effective in reducing quenching of fluorescence emissions of PAH in methanol/water mobile phase. We attributed the observed increase in fluorescence signal to the catalytic removal of dissolved oxygen in the mobile phase. The fluorescence emission of pyrene, for example, increased almost 8-fold whether the catalyst column was inserted before the sample injection valve or before or after the analytical column. As a result of this discovery, we have since used a catalyst column between the pump and the sample injection valve in all our reversed-phase HPLC systems using methanol/water mobile phase.

Estimation of Catalyst Efficiency. The reduction of the nitro to the amino group changes the polarity of the analyte species. Both species may be separable chromatographically if their polarity difference is large enough. The separation may be accomplished by adding a second analytical ODS column, identical to the first analytical ODS column, at the outlet of the catalyst column. Since, as noted earlier, the analyte species are practically unretained in the catalyst column, the addition of a second ODS column merely doubles the retention volume of any unreduced analyte. Quantitation of the unreduced analyte with and without the catalyst in line provides a convenient method for estimating the reducing efficiency of the catalyst.

Figure 3 illustrates the points discussed above. Doubling the column length locates the expected retention volume of any unreduced 1-nitropyrene through the ODS–catalyst–ODS columns (A and B). No measurable UV peak at this retention volume suggests that the reduction of 1-nitropyrene approaches 100%. Only one main reduction product, 1-aminopyrene, is apparent from the simultaneous UV and fluorescence profiles, and it is clearly more polar than 1-nitropyrene (C). Since the mass (200 ng) of 1-nitropyrene injected onto the column was already 400–1000 times greater than that normally observed in real samples, no attempts were made to determine the maximum reductive capacity of the catalyst column. Reduction efficiencies approaching 100% were likewise observed for the nitro-PAH that are listed with on-column detection limits in Table I.

The 1-aminopyrene peak in Figure 3 is slightly broadened and shows some tailing. Three factors are mainly responsible for this observed peak shape: (1) the extra column broadening due to the void volumes of the catalyst and the second ODS columns and the volume of the connecting tubings, (2) the kinetics of chemical reactions in the catalyst column, and (3) the difference in the elution characteristics of 1-nitropyrene and 1-aminopyrene on the ODS column; i.e., the elution of 1-nitropyrene is retarded in the first ODS column, but speeds up once it is transformed to 1-aminopyrene by the catalyst, in the second ODS column.

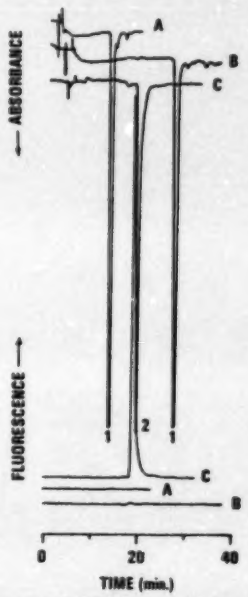

Figure 3. Simultaneous UV (254 nm) and fluorescence (360 nm excitation, 430 nm emission) profiles illustrating the efficiency of conversion of 200 ng of 1-nitropyrene to 1-aminopyrene by the Pt/Rh catalyst. Column configurations are as follows: (A) ODS–detector, (B) ODS–ODS–detector, (C) ODS–catalyst–ODS–detector. Both ODS columns are identical Zorbax ODS (4.6 mm i.d. × 15 cm); catalyst column (4.6 mm i.d. × 5 cm) packed with Pt/Rh coated 5-μm alumina; mobile phase, 80% methanol in water at 1 mL/min; peak identities, (1) 1-nitropyrene and (2) 1-aminopyrene.

Application of the Catalyst to Real Samples. The fluorescence spectra of PAH, such as pyrene and fluoranthene, that were passed through an ODS–catalyst column confirmed that these compounds are not chemically transformed by the catalyst. The addition of a second ODS column broadened the PAH peaks slightly but did not affect the peak symmetry. However, compounds other than the nitro-PAH are also chemically transformed upon passage through the catalyst. The fluorescence profiles of oxygenated fractions of diesel particulate extract showed some reduction in peak intensities and sometimes the disappearance of peaks when the catalyst was in line. This indicated that significant reactions did occur (Figure 4). These reactions have not been defined nor has their possible utility been explored.

In some samples, the presence of specific nitro-PAH, was readily detected by comparing their fluorescence profiles with and without the catalyst postcolumn. The presence of the specific nitro-PAH is usually indicated by an enhancement of fluorescence signal. This observation is generally valid if the mobile phase is sufficiently deoxygenated before entering the sample injection valve (e.g., by inserting a catalyst column between the HPLC pump and the sample injection valve). Without deoxygenation, an enhanced fluorescence signal upon passage of a PAH through the catalyst could be mistakenly attributed to the reduction of some nitro-PAH. Those samples that contain high concentrations of PAH and trace amounts of nitro-PAH generally required semipreparative normal-phase separation on silica gel either by open column chromatography or by HPLC. We have used both procedures for getting enriched nitro-PAH fractions for qualitative analysis.

Figure 5 shows profiles of an enriched fraction of a diesel particulate extract containing 3- to 5-ring nitro-PAH that was

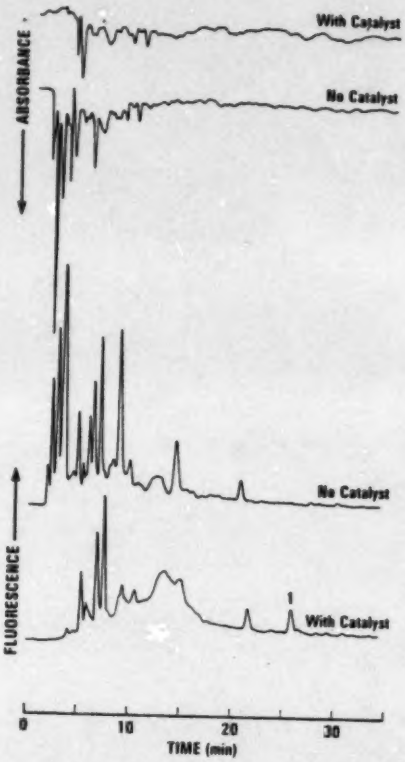

Figure 4. Simultaneous UV (254 nm) and fluorescence (360 nm excitation, 430 nm emission) profiles illustrating the effect of the catalyst on the highly polar components of a diesel particulate extract. Conditions are as follows: analytical column, 4.6 mm i.d. × 25 cm Zorbax ODS; gradient program, 75–100% methanol in water in 30 min at 1 mL/min. Peak 1 is 1-aminopyrene.

obtained by using open column chromatography. The fluorescence detector was optimized to detect 1-aminopyrene (Figure 5a) and 9-aminoanthracene (Figure 5b). Note the disappearance of the polar peak (retention time ~2.5 min) and the reduction of the peak heights of the prominent peaks in the absorbance profiles in the presence of the catalyst. The slight offset in retention volumes of corresponding prominent peaks in the absorbance profiles with and without the catalyst is due to the void volume of the catalyst column. The fluorescence profiles illustrate rather dramatically the high selectivity and sensitivity that can be gained by reducing the nonfluorescent nitro-PAH to aromatic amines. 9-Aminoanthracene, 1-aminopyrene, and 6-aminobenzo[*a*]pyrene were positively identified in this sample by their retention times and fluorescence spectra.

Figure 6a shows the isolation of a 1-nitropyrene-rich fraction, F_3, in a diesel particulate extract using normal-phase HPLC. A step gradient was used to speed up the isolation of this fraction. The fractionation procedure is a variant of a semipreparative elution scheme developed in our laboratory (*39*) and subsequently modified and improved by Schuetzle and his group at Ford Motor Co. (*5*, *32*). The types of compounds in fractions F_1–F_4 have been identified by Schuetzle (*5*). Figure 6b shows a chromatogram of fraction F_3 monitored at the optimum detection wavelength for 1-aminopyrene.

Routine Determination of Specific Nitro-PAH. Semipreparative normal-phase fractionation is tedious and time-consuming for quantitative routine monitoring applications. This is especially true when the number of samples to be analyzed is large or when only one or two nitro-PAH are to be monitored. In some cases fractionation may not be practical because the amount of sample is very limited.

Table II. 1-Nitropyrene in Soluble Organic Fraction (SOF) of Particles in Exhaust Emissions of In-Use Gasoline- and Diesel-Powered Vehicles under Simulated Driving Conditions[a]

	particle mass, mg	SOF, % of particle mass	1-nitropyrene, μg/g of SOF
	Gasoline		
1970 van wagon			
FTP	2182	25.4	6.1
LA-4	2316	17.0	5.6
HWFET	2604	14.7	17.0
CUE	1998	26.0	10.9
NYCC	619	24.4	3.7
1977 van wagon			
FTP	272	47.9	114
LA-4	190	41.6	77.2
HWFET	1648	71.3	3.4
CUE	916	53.7	14.6
NYCC	333	19.3	173
1977 passenger car			
FTP	83	42.3	5.1
LA-4	150	55.0	90.0
HWFET	1089	11.5	36.8
CUE	406	30.4	10.9
NYCC	89	36.8	12.7
1981 passenger car			
FTP	751	8.9	21.2
LA-4	168	15.2	22.6
HWFET	822	8.2	31.3
CUE	472	10.2	13.6
NYCC	122	19.7	29.1
	Diesel		
1978 passenger car			
FTP	5110	11.4	100
HWFET	2860	14.5	82.5
NYCC	939	16.1	111
45SS	1265	23.1	26.3
1980 passenger car			
FTP	2925	25.3	58.5
HWFET	2820	20.9	75.2
NYCC	520	21.7	63.8
45SS	1280	24.5	15.2

[a] Concentration values are based on a single determination.

The analytical system we have developed for routine analysis of a specific nitro-PAH is a simple but powerful version of an on-line multidimensional HPLC system (*37*, *38*). Conventional multidimensional HPLC relies mainly on column selectivities to achieve the desired separation. The incorporation of a catalyst column in line introduces chemical transformation as another important selectivity factor in analyte separation. This greatly simplifies the selection of analytical columns and mobile phases. A single mobile phase and identical primary and secondary columns may be used to achieve highly selective separation.

The analytical system is shown in Figure 1. It is basically an extended application of the coupled-column technique we used earlier in estimating the efficiency of the catalyst for reducing different nitro-PAH. The design of the analytical system was based on the experimental observation that nitro-PAH elutes slower than its amine derivative on the ODS column.

The sequence of analytical steps performed by the system is as follows: (1) the specific nitro-PAH is initially separated on the ODS column and immediately reduced to amino-PAH on the catalyst column; (2) the whole fraction containing the specific amino-PAH is injected onto the second ODS column; (3) the specific amino-PAH is separated from the fraction contaminants on the second ODS column and selectively

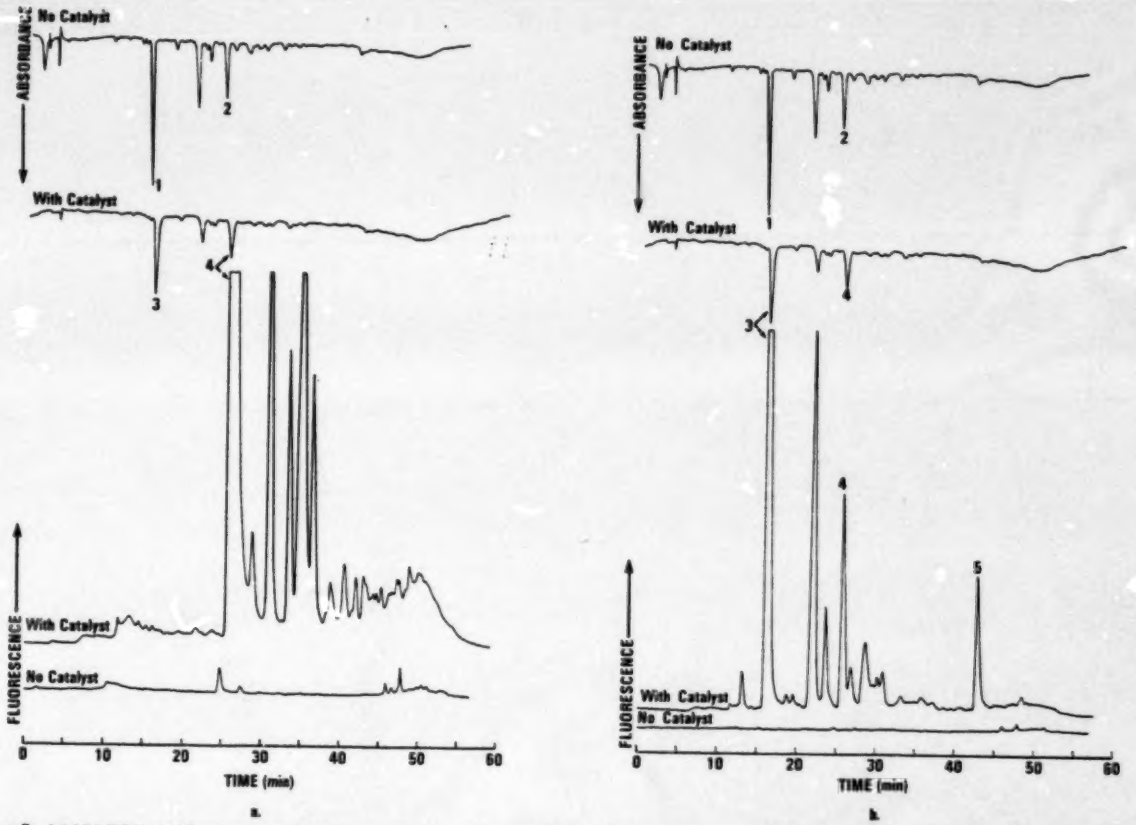

Figure 5. (a) UV (254) and fluorescence (360 nm excitation, 430 nm emission) profiles of a moderately polar fraction of a diesel particulate extract. Same conditions as in Figure 4. Peak identities are (1) 9-nitroanthracene, (2) 1-nitropyrene, (3) 9-aminoanthracene, and (4) 1-aminopyrene. (b) Same sample and conditions as in Figure 5a, except excitation/emission wavelengths = 263/505 nm. Peak identities are (1) 9-nitroanthracene, (2) 1-nitropyrene, (3) 9-aminoanthracene, (4) 1-aminopyrene, and (5) 6-aminobenzo[*a*]pyrene.

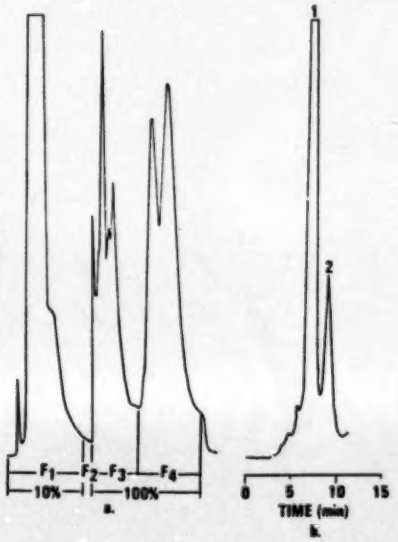

Figure 6. (a) Sample cleanup by semipreparative normal-phase HPLC fractionation of a diesel particulate extract on a silica gel (100–200 mesh BioSil A) column (4.6 mm i.d. × 25 cm). Fluorescence detection 313-nm excitation, 415-nm cutoff filter emission. Flow rate is 1.5 mL/min. (b) Fluorescence profile (360 nm excitation, 430 nm emission) of PAH-free fraction, F_3, containing 1-nitropyrene through the ODS–catalyst columns. Peak identities are (1) 1-aminopyrene and (2) unknown.

detected by fluorescence; and (4) the ODS–catalyst column is cleaned and equilibrated for the next sample injection. The analytical system is automated once the sample is injected.

The injection of the fraction onto the second ODS column is normally started 1 min before the elution of the amino-PAH from the catalyst column and extends 1–2 min beyond the end of the peak. These time events are predetermined from the elution characteristics of a standard amino-PAH peak. This ensures that the amino-PAH fraction is quantitatively injected onto the second ODS column. From our experience, this injection window is generally sufficient to accommodate normal variations in the retention volume of the amino-PAH in the ODS–catalyst column due to effects of various matrices. Even though the total volume of the amino-PAH fraction could be as much as 6 mL, peak broadening of the amino-PAH peak in the second ODS column is not generally significant. This is principally due to the fact that the analyte is not subjected to compositional changes in the mobile phase during its elution through the second ODS column.

As configured, the ODS–catalyst columns are completely isolated from the mobile phase during the separation of the specific amino-PAH on the second ODS column. In principle, each nitro-PAH remaining on the ODS–catalyst columns can be selected for separation and detection by repeating steps 1–3. This is indeed possible. We have demonstrated in our laboratory, for example, that 9-nitroanthracene and 1-nitropyrene can be selectively detected from a single injection of a complex organic sample. This inherent capability of the analytical system can be fully exploited if the fluorescence detector can be programmed to reset with precision such parameters as excitation and emission wavelengths, slit widths, range and sensitivity, and background zero for every nitro-PAH to be determined. For quantitative routine analysis, it is simpler to make one component determination per sample

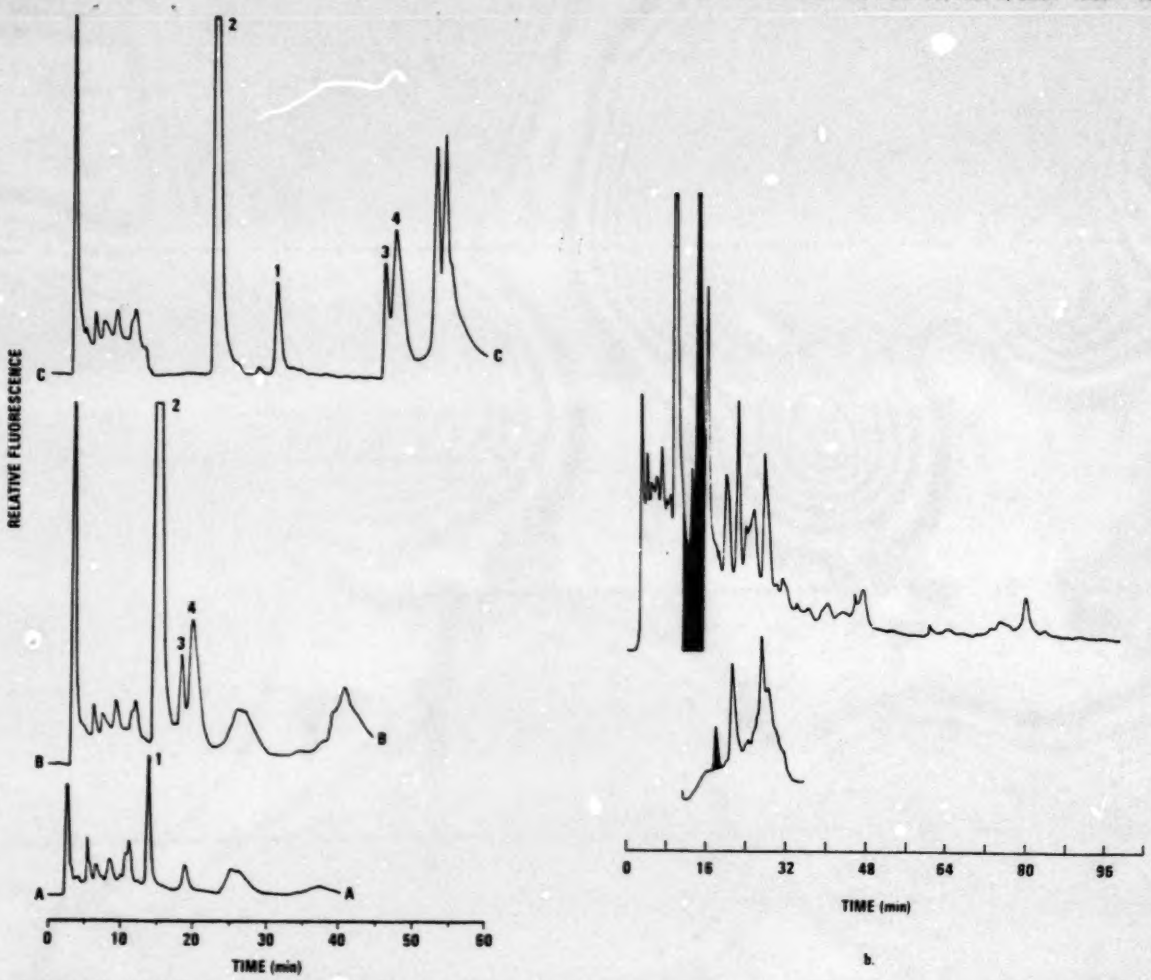

Figure 7. (a) Selective separation and detection of 1-nitropyrene in 25 μg of unfractionated diesel particulate extract; fluorescence detection at 360 nm excitation and 430 nm emission: (A) through the ODS column, (B) through the ODS–catalyst column, and (C) through the analytical system. Peak identities are (2) 1-aminopyrene, (1) fraction contaminant, and (3 and 4) unknowns. (b) Selective separation and detection of 9-nitroanthracene in a raw diesel particulate extract: fluorescence detection at 263 nm excitation and 505 nm emission. Shaded area in upper profile represents fraction injected onto the second ODS column; bottom profile shows resolution of the fraction on the second ODS column; shaded peak is 9-aminoanthracene corresponding to 300 pg of 9-nitroanthracene. Profiles shown were obtained under isocratic conditions, 75% methanol at 1 mL/min.

injection if the detector is not programmable.

Figure 7a illustrates the different stages in the selective separation and detection of 1-nitropyrene in a raw diesel particulate extract using the analytical system. Trace A (through the ODS column only) shows a contaminant (peak 1) that is completely obscured by 1-aminopyrene (peak 2) when a catalyst postcolumn is inserted (B). Trace C shows the separation of 1-aminopyrene from the contaminant using the column switching program of the analytical system. The 1-aminopyrene peak is narrower in C than in B because a gradient program was used during the separation of the fraction components in the second ODS column. Ordinarily, isocratic elution is used until all fraction components have eluted from the second ODS column. That way, the second ODS column would be ready for the next fraction injection. Note the elution of components 3 and 4 when the mobile-phase flow was switched back through the ODS–catalyst column during the cleanup and equilibration.

Figure 7b demonstrates the capability of the analytical system for isolating and detecting sub nanogram quantities of 9-nitroanthracene in a complex organic matrix. The shaded area in the upper profile represents the fraction injected onto the second ODS column. The shaded area in the bottom profile is 9-aminoanthracene corresponding to 300 pg of 9-nitroanthracene.

Analysis using the analytical system is very repeatable. Five 10-μL manual injections of a 0.1 μg/mL 1-nitropyrene standard gave a relative standard deviation of 2.7%. An experiment to assess the accuracy of the analytical system in the determination of 1-nitropyrene that was spiked into a raw diesel extract was performed. A recovery of 101.6% of the spiked 1-nitropyrene (500 pg/mL in the raw extract) was obtained.

We have used this analytical system for routine determinations of 1-nitropyrene in particulate emissions from gasoline- and diesel-powered vehicles (*23*, *40*). Some typical results are summarized in Table II.

Our analytical system, which uses Pt/Rh catalyst for reducing the nitro-PAH, has decided advantages over other methods employing the derivatization approach. It does not need consumable special reagents other than the methanol or aqueous methanol already used as the mobile phase. The catalyst column has an indefinite lifetime; i.e., the Pt/Rh is not consumed. Minimum sample is wasted since the sample is derivatized as needed and only in amounts necessary to detect the analyte. Analytical precision is increased since there

is no intermediate sample handling with concomitant errors. The loss of derivatized product is greatly minimized since the reduction takes place in a closed system. In addition, cleaner reaction products are formed since the reactions take place in a substantially simpler matrix; i.e., initial cleanup has already been accomplished by the first analytical ODS column. Lastly, the entire analytical procedures can be readily automated.

ACKNOWLEDGMENT

We thank Lynn H. Wright of U.S. EPA for providing solids probe MS analysis of the nitro-PAH and amino-PAH mentioned in this report and Bruce A. Tomkins of Oak Ridge National Laboratory for his critical comments and many helpful suggestions to improve the manuscript.

Registry No. Pt, 7440-06-4; Rh, 7440-16-6; 1,5-dinitronaphthalene, 605-71-0; 1-nitronaphthalene, 86-57-7; 2-nitrophthalene, 581-89-5; 1,3,6,8-tetranitropyrene, 28767-61-5; 2-nitrofluorene, 607-57-8; 9-nitroanthracene, 602-60-8; 2-nitroanthracene, 3586-69-4; 1,3,6-trinitropyrene, 75321-19-6; 1,6-dinitropyrene, 42397-64-8; 9-methyl-10-nitroanthracene, 84457-22-7; 1,8-dinitropyrene, 42397-65-9; 1-methyl-9-nitrophenanthrene, 87517-97-3; 8-nitrofluoranthene, 13177-32-7; 1-nitropyrene, 5522-43-0; 3-nitrofluoranthene, 892-21-7; 1,3-dinitropyrene, 75321-20-9; 4-methyl-3-nitropyrene, 88535-51-7; 6-nitrochrysene, 7496-02-8; nitrobenz[*a*]anthracene, 96529-01-0; 6-nitrobenzo[*a*]pyrene, 63041-90-7.

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RECEIVED for review March 5, 1984. Resubmitted January 24, 1986. Accepted March 7, 1986. This report has been reviewed by the Atmospheric Sciences Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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